Reduced Adipocyte Insulin Sensitivity in Caucasian and Asian Subjects with Coronary Heart Disease

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The association between insulin resistance and coronary heart disease (CHD) is strong in the British Indian-Asian population. Adipocyte metabolism may contribute to both insulin resistance and CHD. We examined insulin-stimulated glucose uptake in adipocytes and *in vivo* insulin sensitivity using the fasting insulin resistance index (FIRI) in 60 subjects (45 Caucasian and 15 Asian) with CHD and 30 Caucasian subjects without CHD. In 25 CHD subjects (18 Caucasian and 7 Asian), the relationship between adipocyte insulin sensitivity and non-esterified fatty acid (NEFA) suppression to oral glucose was examined. Compared with controls, the CHD subjects had higher values of fasting insulin [51 (46 to 54) pmol l^{-1} vs 36 (31 to 41) pmol l^{-1} $p < 0.01$] and FIRI [1.65 (1.5 to 1.79) vs 1.06 (0.89 to 1.23), $p < 0.01$]. Among the CHD subjects, the Asians had higher values than Caucasian [insulin 58 (48 to 67) pmol l^{-1} vs 48 (44 to 53) pmol l^{-1} $p < 0.01$, FIRI 1.89 (1.44 to 2.13) vs 1.62 (1.4 to 1.79), $p < 0.01$)]. Insulin-stimulated glucose uptake in adipocytes was lower in the CHD than control subjects [56 (50 to 62) vs 115 (75 to 132) attomol $min^{-1}.mm^2$, $p < 0.05$], being most reduced among the Asians. It was positively correlated with postprandial NEFA suppression and negatively with insulin release. In conclusion, abnormalities of adipocyte function and insulin sensitivity occur in CHD and may contribute to its aetiology. © 1998 John Wiley & Sons, Ltd.

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Introduction

Epidemiological and clinical studies point to insulin resistance being an independent risk factor for coronary heart disease (CHD).[1] The association between insulin resistance and CHD is particularly apparent among Indian Asian subjects, who are at greater risk for both conditions than the white Caucasian population.[2] It is possible that a common metabolic abnormality underlies the pathogenesis of the insulin resistant state and CHD. Recently it has been proposed that it is the increased release of adipocyte NEFA (non-esterified fatty acid) in insulin-resistant states that predisposes to this atherogenic risk.[3] In muscle, the main tissue determinant of whole body insulin sensitivity, circulating NEFA, reduces insulin sensitivity by competing with glucose as a cellular substrate.[3] In adipose tissue, insulin-stimulated glucose uptake is also reduced and adipocyte NEFA release increased.[4] We have previously shown that in patients with CHD there is a correlation between insulin stimulated glucose uptake of isolated adipocytes and insulin sensitivity as measured during hyperinsulinaemic euglycaemic clamps.[5] In the present study we examined insulin-stimulated glucose uptake in adipocytes and made an assessment of whole body insulin sensitivity in cardiac surgical patients with and without established CHD. The handling of postprandial NEFA metabolism was also investigated in a sub-group of the CHD patients. Results were analysed to explore potential metabolic atherogenic differences between the CHD patients and controls as well as between the Asian and Caucasian CHD patients.

Patients and Methods

Sixty consecutive patients, 45 Caucasian and 15 Asian, with angiographically proven CHD, who were awaiting coronary artery by-pass grafting surgery at the hospital, were invited to participate in the study. A control group

Abbreviations: CHD coronary heart disease, NEFA non-esterified fatty acids, DMEM Dulbecco's Modified Eagle Medium, KRP Kreb's Ringer Phosphate, BSA bovine serum albumin, HDL high density lipoprotein, LDL low density lipoprotein, CI confidence limit, FIRI fasting insulin resistance index, OGGT oral glucose tolerance test, HSL hormone sensitive lipase, LPL lipoprotein lipase

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was recruited from the cardiac surgery list of patients awaiting valve surgery. Of the 45 consecutive control patients invited to participate, 30 agreed and all had had normal coronary angiography. All subjects and controls were between 18 and 70 years old; none were diabetic or had any other active endocrine or metabolic medical conditions. None of the subjects or controls had made changes to their habitual diet prior to the study. This study had been approved by the Hammersmith Hospital NHS Trust's Research & Ethics Committee. All participants gave informed consent.

In the week prior to surgery the patients were seen on a metabolic ward where demographic (including drug and smoking history) and anthropometric data were recorded. Fasting blood samples were taken for plasma glucose, insulin, and lipid profiles. At the time of cardiac surgery, a 1–5g pre-sternal fat biopsy was taken for the *in vitro* measurement of insulin-stimulated glucose uptake in isolated adipocytes. According to previously described techniques,[5] a 1–5g biopsy was obtained, using a scalpel and without cauterization, subcutaneously from the mid-point of the sternal incision. Following immediate transfer of the biopsy to the laboratory in Dulbecco's Modified Eagle Medium (DMEM) containing 5 % bovine serum albumin (BSA), glucose uptake in isolated adipocytes was quantified using modifications to previously described and validated methods.[4,6,7] The adipocytes were initially isolated by finely mincing the biopsy and incubating it for 45 min in DMEM buffer + 5 % BSA containing collagenase (1mg per 0.5g of tissue) in a vibrating water bath at 14 cycles min^{-1}. The cells were then filtered through a 400 nm mesh and washed three times in glucose free buffer with 5 % BSA (each wash involving five inversions). At no time were the adipocytes left to stand for more than 5 min without being agitated. The cells were then left to incubate in the vibrating water bath for a further 2 h in 95 % oxygen saturated glucose free KRP buffer with 5 % BSA, after which the cells were concentrated. The *in vitro* studies were then performed on approximately 30 000 isolated adipocytes ($\cong$ 25 μl), with the cells kept in the vibrating water bath throughout. The adipocytes were initially incubated for 45 min in 500 μl of KRP + 5 % albumin buffer in the presence of 1 nM insulin (Sigma UK) (see below) before the addition of 300 nM of the non-metabolizable radiolabelled glucose analogue (0.1 μCi of 2-deoxy-[U-14C]-D-glucose) and a further 15 min of incubation prior to centrifugation through 500 μl of silicon oil. Glucose uptake was calculated following liquid scintillation counting of the radiolabelled glucose tracer in the isolated adipocytes. Cells were manually counted in a 1 in 100 dilution of the cell concentrate with the use of a haemocytometer (with a 200 μm gap) and cell size, diameter, and surface area were measured on 50 cells using a Cue 2 cell counter (Olympus Ltd, London, UK). The optimal concentration of insulin for this *in vitro* assay was established in the first 13 (7 CHD and 6 controls) initial studies in which isolated adipocytes were incubated with insulin concentrations ranging from 0.001, 0.01, 0.1, 1, 10 nM using the above protocol.

The suppression of NEFA following oral glucose was examined in the 25 CHD (18 Caucasian and 7 Asian) patients who agreed to have a 75 g oral glucose tolerance test (OGGT) prior to their by-pass surgery. During the OGTT 10 ml blood samples were taken at time −30, 0, 30, 60, 90, 120 min and immediately centrifuged and separated prior to storage at −20 °C for later analysis. The suppression of NEFA during the OGTT was calculated by subtracting the integrated area of the NEFA obtained from the four measurements during the OGTT from the two basal values, with NEFA suppression expressed as an incremental area under the curve.

Blood samples were assayed for plasma glucose, cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides using standard colorimetric techniques (Boeringer, Lewes, East Sussex, UK), and insulin by radioimmunoassay as previously described.[8] This assay had an intra- and inter-assay variation of 7 % and 10 %, respectively, with a lower detection level of <5 $pmol^{-1}$ with 95 % confidence. The assay was non-specific and cross-reacted with other immuno-like insulin molecules. Analysis for NEFA was carried out using a WAKO colorimetric kit (Wako Chemicals Ltd, Neuss, Germany). *In vivo* insulin sensitivity was assessed using the FIRI ((fasting glucose mmol l^{-1} × fasting insulin mU l^{-1}) ÷ 25) as previously validated.[9,10]

Statistical Analysis

Results are presented as means with 95 % confidence intervals (CI). For parameters that were not normally distributed (BMI, cell diameter, cell surface area, and insulin-stimulated glucose uptake), data were logarithmically transformed and summarized as the geometric mean with 95 % confidence intervals (CI). Using Student's *t*-test and chi-squared analysis for comparisons between two groups and analysis of variance when analysing more than two groups. Correlation was by univariant linear regression. A significant difference was taken at the 5 % level.

Results

The CHD patients undergoing by-pass surgery were of similar age and body mass index (BMI) to control subjects without CHD and both groups had similar proportions of men to women. The use of diuretics and β-blockers did not differ between the two groups and no subject in either group was taking a lipid lowering drug (Table 1). The number of current and ex-smokers was also similar between the CHD and control groups as was the number of hypertensive subjects.

The CHD group compared to the non-CHD group had higher fasting plasma glucose (6.1 (5.8–6.5) mmol l^{-1} vs 5.3 (5.1–5.8) mmol l^{-1}), insulin (51 (46–54) pmol l^{-1} vs 36 (31–41) pmol l^{-1} and total cholesterol values (6.4

Table 1. Demographic details of control and CHD patients

Parameter	Controls		CHD		Caucasian CHD		Asian CHD	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
Number	30		60		45		15	
Sex (*n* (%))	18 M (60)		45 M (75)		30 M (67)		15 M (100)	
Age (yr)	60	57 to 63	61	59.4 to 62	62	64 to 59	58	54 to 61
BMI (kg m^{-2})[d]	28.1	27.7 to 28.5	28.6	28.1 to 29.1	28	30 to 27	29	31 to 27
Smokers and ex-smokers *n* (%)	20 (67)		47 (78)		36 (80)		11 (73)	
Hypertensives *n* (%)	6 (20)		18 (30)		12 (27)		6 (40)	
β-blocker *n* (%)	4 (13)		15 (25)		11 (24)		4 (26)	
Diuretics *n* (%)	6 (20)		12 (20)		8 (18)		4 (27)	
Cholesterol ($mmol^{-1}$)	5.3[a]	5.1 to 5.5	6.4	6.1 to 6.7	6.1	5.7 to 6.5	6.5	5.9 to 7.1
Triglycerides ($mmol^{-1}$)	1.54	1.27 to 1.81	1.69	1.37 to 2.01	1.71	1.21 to 2.21	1.51	1.2 to 1.8
LDL cholesterol ($mmol^{-1}$)	3.9[a]	3.7 to 4.1	4.6	4.3 to 4.9	4.4	3.9 to 4.9	4.7	4.3 to 5.1
HDL cholesterol ($mmol^{-1}$)	1.05	1.0 to 1.1	1.03	0.98 to 1.08	1.06	0.96 to 1.16	0.98	0.7 to 1.26
Fasting NEFA ($mmol^{-1}$)	0.3	0.25 to 0.35	0.27	0.24 to 0.3	0.24	0.22 to 0.26	0.28	0.20 to 0.36
Fasting glucose ($mmol^{-1}$)	5.3[a,b]	5.1 to 5.8	6.1[a]	5.8 to 6.5	6.3[b]	5.9 to 6.7	5.8	5.1 to 6.1
Fasting insulin ($pmol^{-1}$)	36[a,b]	31 to 41	51[a]	46 to 54	48[c]	44 to 53	58[b,c]	48 to 67
FIRI	1.06[a,b,c]	0.89 to 1.23	1.65[a]	1.5 to 1.79	1.62[b]	1.4 to 1.79	1.89[b]	1.44 to 2.13
Cell diameter (μm)[d]	74[a]	66 to 81	94	98 to 89	94	99 to 89	97	89 to 105
Cell surface area (μm^2)[d]	18074[a]	14346 to 21801	28677	26230 to 31124	28744	25950 to 31538	30689	25684 to 35683

[a]Significant difference between CHD and controls <0.01.
[b]Significant difference between controls and Asian or Caucasian CHD subjects $p < 0.05$.
[c]Significant difference between Asian and Caucasian subjects with CHD.
[d]Data presented as the geometric mean.

(6.1–6.7) mmol l^{-1} vs 5.3 (5.1–5.5 mmol l^{-1}) $p < 0.01$ in all instances). Insulin resistance, assessed by the FIRI method, was higher in the CHD patients than the controls (1.65 (1.5–1.79) vs 1.06 (0.89–1.23), $p < 0.01$). Of the CHD patients, the Asian subjects had higher fasting insulin values than the Caucasian patients (58 (48–67) pmol l^{-1} vs 48 (44–53) pmol l^{-1} $p < 0.01$) and higher FIRI values (1.89 (1.44–2.13) vs 1.62 (1.4–1.79), $p < 0.01$).

The FIRI value was not significantly different in the 10 CHD subjects taking both a diuretic and β-blocker to that in the 12 CHD subjects on neither drug (1.75 (CL 1.56–1.94) vs 1.69 (CL 1.43–1.95)). There was no significant difference in the FIRI value for the 15 CHD subjects currently smoking and the 13 CHD subjects who had never smoked (1.78 (CL 1.68–1.87) vs 1.67 (CL 1.56–1.78)).

Adipocyte cell diameter and cell surface area were significantly higher in the CHD group than their controls (94 (98–89) μm vs 74 (66–81) μm and 28074 (26230–31124) μm^2 vs 18074 (14346–21801) μm^2, respectively $p < 0.01$); see Table 1.

The adipocytes of 13 subjects (7 with CHD and 6 without CHD) were examined under basal conditions and following incubation with increasing concentrations of insulin, 0 to 10 nM. When glucose uptake was expressed as a function of cell area for each insulin concentration, the subjects with CHD had lower glucose uptake than the controls (Figure 1). The maximum insulin-stimulated glucose uptake in adipocytes was observed when cells were pre-incubated with 1nM insulin (Figure 1). For the controls there was a significant increase above basal adipocyte glucose uptake at all concentrations above 0.01nM insulin ($p < 0.01$). Similarly for the CHD subjects a significant increase in glucose uptake above basal values was seen with insulin concentrations above 0.1nM insulin. This rise above basal glucose uptake continued up to 1nM insulin, however above 1nM no further increase was seen. This concentration of insulin was therefore used for all subsequent adipocyte studies.

As in the initial pilot study, basal adipocyte glucose uptake in the 30 controls compared to the 60 CHD subjects was significantly higher and this difference became more marked following insulin stimulation [controls 183 (136–225) vs CHD 86 (62–100) attom. $min^{-1}.mm^2$] (Figure 2). Under basal conditions the Asian CHD subjects had a non-significantly lower adipocyte glucose uptake than the Caucasian CHD patients, this difference however became significant following insulin stimulation (Figure 2).

The insulin-stimulated glucose uptake in adipocytes was not significantly different in the 10 CHD subjects taking both a diuretic and β-blocker than for the 12 CHD subjects on neither drug (26 % (CL 18–33) vs 28 % (CL 19–37)). There was no difference in insulin-stimulated glucose uptake in adipocytes in the 15 CHD smokers compared to the 15 CHD subjects who had never smoked (24 % (CL 16–32) vs 31 % (CL 16–46)).

Among the 25 CHD subjects who had an OGTT, a positive correlation was seen between the suppression of NEFA during the 75 g OGGT and the adipocyte insulin-stimulated glucose uptake (linear regression $r = 72$ (0.45–0.87), $p < 0.001$) (Figure 3(a)). By contrast, an inverse correlation was observed between the insulin area under the OGTT curve and the percentage increase in insulin-mediated adipocyte glucose uptake (linear regression $r = -70$ (−0.86–−0.43), $p < 0.0001$) (Figure 3(b)). No relationship however existed between insulin-stimulated adipocyte glucose uptake and the fasting plasma glucose values.

The relationship between a reduced adipocyte insulin-stimulated glucose uptake and reduced NEFA suppression during the OGGT was more marked in the Asian than Caucasian patients, with all 7/7 of the Asian patients

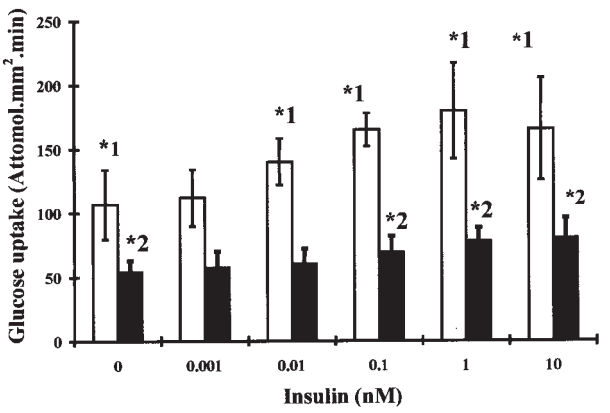

Figure 1. Glucose uptake in isolated adipocytes taken from 6 control (□) and 7 CHD (■) subjects at different concentrations of insulin (mean values with SEM). There is a significant difference within groups (*1 : *1 and *2 : *2) at baseline and at each insulin concentration >0.01nM in the control group and baseline and >0.1nM in the CHD group of at least $p<0.05$. The rise above basal glucose uptake continued up to 1nM insulin, however above 1nM no further increase was seen

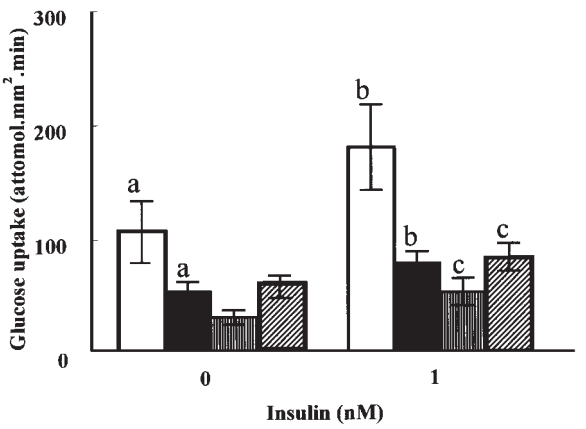

Figure 2. Comparison of basal and insulin (1nM)-stimulated glucose uptake in adipocyte between subjects with (CHD) and without (controls) coronary heart disease where results are expressed by cell surface area mm^2. Studies were performed on 30 controls (■), 60 CHD subjects (□), 15 Asians with CHD (▥), and 45 Caucasians with CHD (▨). The figure demonstrates a significant difference betwen a:a, b:b and c:c of at least $p < 0.05$

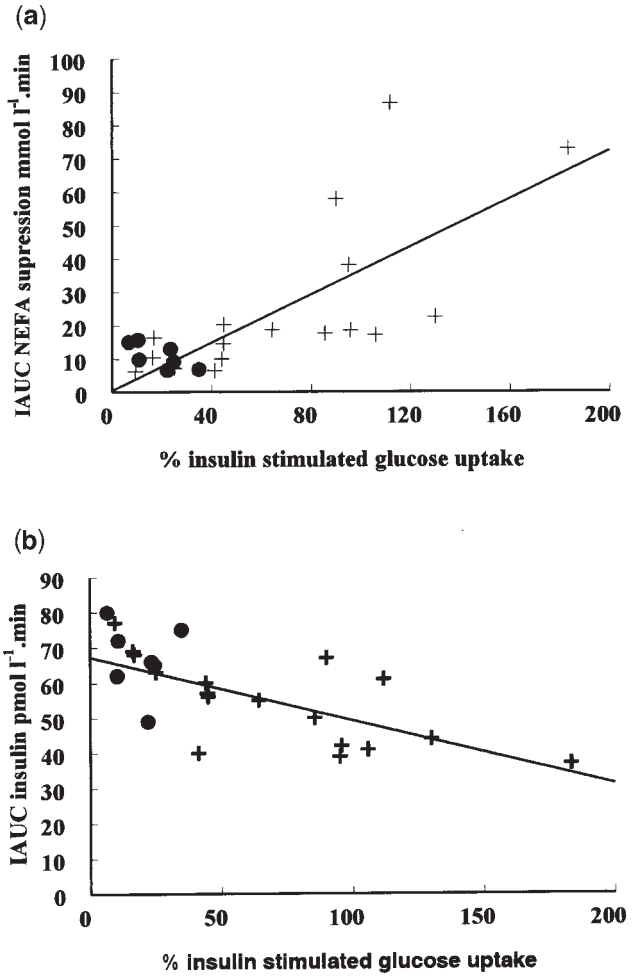

Figure 3. Scatter graphs of (a) the NEFA (expressed as NEFA area under the OGTT curve) and (b) on the Y-axis and adipocyte insulin-stimulated glucose uptake expressed as % increase over basal X-axis in 18 Caucasian (+) and 7 Asians (•) with coronary heart disease

being in the lowest tertile for both adipocyte insulin stimulate glucose uptake and NEFA suppression compared with 8/18 for the Caucasians CHD subjects ($\chi^2 = 4.2$, $p < 0.02$).

Discussion

Numerous clinical and epidemiological studies have shown an association between insulin resistance and coronary heart disease risk.[1,11–13] Abnormalities in fat metabolism provide a potential metabolic mechanism linking the pathogenesis of insulin resistance with coronary heart disease.[14] At a cellular level, adipocytes play an important role in influencing insulin action.[14] The results of the present study lend support to the hypothesis that adipocyte metabolism may be related to the pathogenesis of insulin resistance and coronary heart disease.

We have examined isolated adipocytes taken from sternal subcutaneous fat, which may appear a less optimal site for metabolic studies than the visceral tissue which is often considered the fat deposit most involved in insulin sensitivity.[15] Morphological and functional differences in adipose deposits sites have been described.[16] However, although the net insulin stimulated glucose uptake in visceral fat is greater than in subcutaneous fat, the percentage increase in uptake is similar.[17] A stronger correlation has been observed between truncal subcutaneous fat and insulin sensitivity than retroperitoneal or intraperitoneal fat.[18] We therefore believe that the use of sternal subcutaneous and not visceral fat in this study does not influence our conclusions.

In the present study we have assumed *in vivo* postprandial NEFA suppression to be a marker of insulin's ability to suppress adipocyte NEFA release, which has previously been shown to correlate with adipocyte insulin-stimulated glucose uptake and insulin release to oral glucose.[4] We did not measure *in vitro* adipocyte NEFA release, due to our concerns that the collagenase separation we used to isolate the cells would influence hormone sensitive lipase (HSL) activity by reducing intracellular cAMP. Other techniques, while technically possible, either require larger biopsies[19] or methods so far not validated in our laboratory.[20]

Our data demonstrate functional and morphological changes in subjects with angiographically proven coronary artery disease. Adipocyte insulin-stimulated glucose uptake was consistently lower in subjects with than without coronary artery disease. There was also an increase in the adipocyte cell surface area in subjects with coronary artery disease. Both reduced adipocyte insulin-stimulated glucose uptake and an increased adipocyte cell surface area have been reported to be associated with reduced whole body insulin sensitivity as assessed by the hyperinsulinaemic euglycaemic clamp.[5,21] The finding that the subjects with coronary artery disease in our study had higher fasting insulin resistance indices, FIRI,[22] than their controls is consistent with the clinical and epidemiological literature. The use of the FIRI provides a simple method of assessing *in vivo* insulin sensitivity and has been shown to correlate well with the euglycaemic clamp when applied to glucose tolerant subjects.[22]

Our results could not be explained by the concurrent use of two commonly prescribed hypotensive agents, β-blockers and diuretics, which are known to increase insulin resistance,[23–26] and our findings could not be explained by smoking also associated with reduced insulin sensitivity.[27]

We have here confirmed our previous work in CHD subjects suggesting that insulin-stimulated glucose uptake in isolated adipocytes is correlated with *in vivo* insulin sensitivity.[5] The present study extends these original observations by showing that both adipocyte and *in vivo* insulin sensitivity are related to postprandial NEFA suppression. These findings suggest that adipose tissue from patients with CHD is more resistant to insulin than that from individuals without CHD. Normal postprandial suppression of circulating NEFA is dependent on insulin, which inhibits hormone sensitive lipase (HSL), thereby

reducing NEFA release from adipose tissue, while stimulating lipoprotein lipase (LPL), an enzyme which facilitates NEFA clearance.[28] The impaired suppression of postprandial NEFA in the coronary artery disease subjects occurred despite exaggerated insulin responses during the OGTT. The observed negative correlation between adipocyte insulin-stimulated glucose uptake and the insulin area under the OGTT curve provides further confirmation that the adipocytes from the CHD patients are more insulin resistant than from their controls. Our data are consistent with the hypothesis that adipose tissue has a primary role in the aetiology of insulin resistance[14] and describes a metabolic phenotype among patients with coronary artery disease, which includes whole body insulin resistance and functional and morphological changes in adipocyte which are themselves more insulin resistant.

In keeping with the literature,[2] the present study demonstrated that the Asian subjects with coronary artery disease are more insulin resistant than the Caucasians. The Asian patients had higher FIRI and fasting insulin values than the Caucasians or the controls free of coronary disease. In addition, reduced adipocyte insulin-stimulated glucose uptake was more marked in the Asian than the Caucasian subjects with CHD and the impairment of post-prandial NEFA suppression was greater.

The postprandial relationship between adipocyte insulin-stimulated glucose uptake and NEFA suppression, we believe, supports a metabolic link between insulin resistance and CHD, combining a detrimental effect of high circulating postprandial NEFA concentrations decreasing glucose oxidation and utilization, while increasing hepatic glucose and very low-density lipoprotein production.[3] This atherogenic metabolic milieu is further compromised by high circulating postprandial NEFA concentrations decreasing hepatic insulin clearance which adds to the peripheral hyperinsulinaemia.

In conclusion, we have shown in an unselected surgical outpatient population of Caucasian and Asian subjects with coronary artery disease that adipose tissue is both insulin resistant to glucose uptake and NEFA suppression, with the greatest resistance being observed in the Asian subjects. We suggest that these abnormalities in adipocyte function are relevant to the aetiology of coronary heart disease and help to explain ethnic differences in the severity and prevalence of CHD.

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